

Bundesinstitut für Risikobewertung (BfR) • Postfach 33 00 13 • D - 14191 Berlin

Bundesinstitut für Risikobewertung  
Postfach 33 00 13  
D - 14191 Berlin  
Tel. 0 30 - 84 12 - 0  
Fax 0 30 - 84 12 - 47 41  
bfr@bfr.bund.de  
www.bfr.bund.de

Neepe Y Choksi, Ph.D.  
Raymond Tice, Ph.D.  
ILS Inc. Co. NICEATM  
NIEHS  
MD EC-17, P.O. Box 12233  
Research Triangle Park NC 27709  
**USA - by Air Mail**

Ihre Zeichen und Nachrichten vom 18.06.2004	Gesch.-Z.: Bitte bei Antwort angeben	Tel.-Durchwahl/Fax 2270	Datum 09.07.2004	Org.-Einheit/Ansprechpartner FGr. 91/ZEBET
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## HET-CAM Test: Background Review Document (BRD)

Dear Neepe and Ray,

please excuse that due to a high workload and the OECD Workshop on Prediction Models/Data Interpretation Procedures I have not been able to respond any earlier.

I am quite happy with the HET-CAM draft BRD. However, after discussing the BRD with Manfred Liebsch, I realised that I have made some comments in my presentation that may not have been correct or misleading. Please allow me, therefore, to provide you with additional information on the following topics.

1. The official French test guideline for safety testing cosmetics for eye irritation properties (attachment 1, please excuse the poor quality, you may be able to find a better copy) . As you can see since Nov. 29, 1996 the HET-CAM assay is an official method for cosmetics testing in France.
2. A copy of pg. 1 of EU Directive 86/609/EEC on the protection of experimental animals (attachment 2). In Article 2 (a) in the definition of an animal under the Directive "foetal or embryonic forms" are excluded. Thus, experiments on embryonated chicken eggs may be conducted under the current EU legislation.
3. A copy of a publication by Martin Rosenbruch (Bayer AG, Germany ) ALTEX 14, 111-113 in German with an English summary "The sensitivity of chicken embryos in incubated eggs" (attachment 3). The author is referring to Hamburger and Hamilton (1951) and to the book "Development of the Avian Embryo" (Freeman and Vince 1974) and claims that the vessels of the yolk sac and of the chorion allantois membrane do not contain sensitive nerves.
4. A copy of pg.765-767 of the ATLA publication of the validation study of the HET-CAM test in Germany (Spiellmann et al., ATLA 24,741-858, 1996) (attachment 4).
5. A copy of a page of my presentation at the OTWG meeting in April 2004 in Washington DC (attachment 5), in which strong irritating chemicals were classified with the endpoint

"mtc 10" and my conclusion was " not sufficient".

Please allow me to comment on the documents #4 + #5. In the publication of the HET-CAM validation study the results of classifying R41 (severely irritating) chemicals using only mtc10 for 142 "new" chemicals are given in Figure 5 on pg. 766. In the text you will see that when using an mtc10 of 174 sec, a specificity of 88% was obtained an overlabelling of 8%. In Figure 6 similar data are presented for the whole group of 189 test chemicals. When an mtc10 of 139sec was used, a specificity of 87% and an over labelling of 9% were obtained.

In a tiered testing strategy a test that is able to identify 88% of the strong eye irritants with an overlabelling of less than 10% is an acceptable test that may be used as the first step of a tiered testing strategy. That was the conclusion of experts in Germany from industry and from the regulatory agencies.

I do have to excuse that in my presentation I have focused too much on the 100% correct classification of severely irritating chemicals. Figures 5+6 show that a mtc10 of 50 sec will provide a 100% correct classification. However - it is quite unfortunate that I did not bring this up in my presentation - an mtc10 of 139 sec will allow to identify severely irritating materials with a specificity of 88% and a 9% chance of over labelling.

In addition, I do have to stress that this is the result that has lead to the acceptance of HET-CAM data for the classification of severely eye irritating materials by the regulatory agencies in Germany.

I hope that my letter will reach you in time and that you will be able to take it into account in the final draft of your BRD of the HET-CAM test.

With the best regards

Dr. med. Horst Spielmann  
Direktor und Professor  
Head of ZEBET

Enclosures !